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Salusin-β mediate neuroprotective effects for Parkinson's disease

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ABSTRACT

Neuropeptides, small peptides found in many mammalian brain, play key roles in communicating with each other to modulate neuronal activity. Here, we reported that endogenous neuropeptide salusin-β has neuroprotective effects on the midbrain dopamine neurons and can be used as an effective therapeutic treatment for Parkinson's disease (PD). We found that the MrgprA1 receptor mediates the neuroprotective effects of salusin-β on the midbrain dopamine neurons. Importantly, intranasal administration of salusin-β in a PD mouse model show the neuroprotection of dopaminergic neurons and increased the survival of midbrain dopamine neurons. Furthermore, inhibition of the salusin-β receptor, MrgprA1, abolished the neuroprotective effects induced by salusin-β. Taken together, these results demonstrate the novel role of salusin-β in the central nervous system and salusin-β can be used as a novel therapeutic to effectively treat PD.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder whose clinical features include tremor, slowness of movement, stiffness, and postural instability [1]. PD is characterized by progressive degeneration of dopaminergic (DA) neurons, the presence of Lewy bodies in DA neurons, and α-synuclein accumulation in the substantia nigra (SN) pars compacta [2]. Although there are drugs that alleviate the symptoms of PD, chronic use of these drugs results in debilitating side effects [3], and none seem to halt the progression of the disease. The etiology of PD remains unknown, but environmental toxins, genetic factors, and mitochondrial dysfunction are thought to be involved.

Salusin-β is a highly conserved 20-amino acid endogenous neuropeptide involved in various functions including vascular inflammation, modulation of the cardiovascular system, cytokine function and oxidative damage [4–6]. In previous studies, salusin-β is expressed in many tissues, such as plasma, myocardium [7] and salusin-β was shown to potentially promote inflammation by increasing activation of the I-κBa/nuclear factor kappa B (NF-κB) signaling pathway [8]. Additionally, salusin-β is abundant in the central nervous system [7], suggesting another function in neurons in addition to its role in inflammatory function. However, a specific role for salusin-β in the central nervous system has yet to be identified.

Recently, we have been reported that a new herbal medicine KD5040 can be used an effective alternative therapy for PD [9,10]. Specifically, we found that treatment with KD5040 upregulated salusin-β expression in the mouse midbrain. In this study, we show that salusin-β mediates the therapeutic effect of KD5040. Furthermore, intranasal administration of the salusin-β peptide reached the midbrain and improved the Parkinsonian phenotypes observed in a mouse model of PD. By inhibiting expression of Mas-related G protein-coupled receptor member A1 (MrgprA1), on which salusin-β may exert its effects, in vitro and in vivo, suggesting that intranasal treatment of salusin-β may be used for the safe and efficient treatment of PD.

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2. Materials and methods

2.1. Culture of primary dopaminergic neurons

Primary midbrain were isolated from wild type E13.5 mouse embryos. The brain were dissected and chopped thoroughly and minced them in HBSS(Gibco). The tissues were incubated in trypsin (2.5%, no EDTA, Gibco) and 0.5 M Ascorbic acid for 20min. Next, the tissues were neutralized into Plating Medium (Neurobasal medium, 2% B27 supplement, 1% L-glutamine, 1% Penisillin/Streptomycin, 10% Fetal bovine serum (FBS), Laminin (2 μg/ml)) and filtrated by 0.45 μm cell strainer (BD Falcon). The cells were seeded on poly-D-lysine (Sigma-Aldrich) coating plates. Next day, the primary DA neurons were changed preparation media with the culture medium (Neurobasal medium, 2% B27 supplement, 0.25% L-glutamine, 1% Penisillin/Streptomycin, 1% FBS, Laminin).

2.2. RNA analysis

All RNA was collected and purified using the eCube tissue RNA mini kit (PhileKorea) following manufacturer's instructions. The total RNA concentration was quantified using Nanodrop spectrophotometer. RNA was reversed transcribed into cDNA using AccuPower® CycleScript RT PreMix (Bioneer). Transcript abundance was determined by quantitive PCR using TOPreal™ qPCR 2X PreMIX (Enzynomics), with primer pairs against Dat, Foxa2, Lmx1b, Nurr1, Girk2, Th, Synapsin, Map2, NeuroD1, MrgprA1, Salusin-β, Vmn1r159, Vnm1r220, Olfr478, Olfr960, and Olfa1238.

2.3. Lentivirus generation

Lentivirus was produced in HEK293T cells, which were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and 1% Penicillin/streptomycin. The cells were transfected with the lentivirus construct shRNA-MrgprA1 vectors using calcium phosphate co-precipitation. The culture medium was replaced 24 h after transfection and the viruses were harvested 72 h later.

2.4. Animals

Male C57BL/6 mice (8 weeks old, 25–28 g weight; Central Laboratories Animal Inc., Korea) were used. All mice were maintained on a 12 h/12 light/dark cycle (light on 9:00 a.m.) and room temperature (23 ± 1 °C) with free access to food and water. All experiments were approved by the Animal Care Committee at Dongguk University. Procedures were performed in accordance with Dongguk University Guideline for the Care and Use of Experimental animals by Dongguk University.

The mice were divided randomly into 3 groups: (1) Control (n = 6; intraperitoneal saline group), (2) MPTP (Sigma-Aldrich) group (n = 5; intraperitoneal 30 mg/kg/day MPTP group), (3) Salusin-β (n = 6; intraperitoneal MPTP plus Salusin-β). Intranasal administration, a non-invasive route for drug delivery, was used to deliver salusin-β (300 nM, dissolved saline; Bio-FD&C) from the nasal cavity into the brain. Salusin-β was administrated intranasally to the mice during MPTP treatment and lasted for 10 days.

To generate MrgprA1 knockdown mouse model, shRNA MrgprA1 lentivirus injected into the substantia nigra at coordinates AP: −3.08 mm, ML: ±1.40 mm, DV: −4.30 mm 10 days after lentivirus injection, the mice were treated with saline and MPTP for 5days.

2.5. Behavioral test

2.5.1. Open field test

The open field test is a very common method to assay locomotor activity levels in mice. Mice were placed in the center of acrylic box (40 × 40 × 40 cm) and video tracking was recorded. Mice were allowed to move without disturbance for 10min. The program Smart (Ver.3.0) was used to measure the patterns of movement and rearing as index of agility and exploration.

2.5.2. Wire hanging test

The Wire Hanging Test is a method to evaluate motor function and deficit in mice model of CNS disorders. Mice were hanged from an elevated wire. The latency times to when the animals ware fall was recorded.

2.6. Brain tissue preparation and immunohistochemistry

Mice were anesthetized with avertin and then perfused with Phosphate Buffered Saline (PBS) and 4% Paraformaldehyde (PFA). The brains were removed and fixed 4% PFA for 24 h before they were transferred to 10% sucrose in PBS for section. The brains were sectioned into 40 μm slices using Leica VT1000 S. For DAB staining, brain were stained with anti-TH antibody (1:500; Millipore) for 24 h at 4° and then incubated anti-mouse Ig HRP (Abcam). The brains were visualized under a Nikon confocal microscope.

2.7. Statistical analysis

All data are presented as the mean ± standard error of three independent experiments. n-values indicate the number of independent experiments performed or the number of individual experiments or mouse. Differences were considered significant at $p < 0.05$. ($^{*}p < 0.05$, #$p < 0.05$, $^{**}p < 0.01$). Significant intergroup differences were analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons and Student's t-test for two-component comparisons after the normal distribution was confirmed.

3. Results

3.1. Identification of salusin-β and MrgprA1 pathway for the protection of dopamine neurons

Previously, we showed the neuroprotective effects of KD5040 treatment in the PD mouse model [9]. Next, the whole-genome analysis used in this study was performed using an Affymetrix GeneChip ST 2.0 and the cluster method to study the similarities and differences in the transcriptome under MPTP treatment and with the addition of KD5040. Gene expression significantly changed (≥2-fold) after KD5040 treatment compared to the gene expression of the MPTP group (Fig. 1A). We identified 35 genes whose expression significantly upregulated (≥2-fold) after KD5040 treatment and Gene Ontology (GO) analysis revealed enrichment of biological processes associated with G protein-coupled receptor signaling pathways in the KD5040 treated midbrain (Fig. 1B). Consistent with this data, we observed a dramatic upregulation of the salusin-β and it's receptor, MrgprA1 then encodes mas related G-protein coupled receptor member A1. The differential expression of these genes was confirmed by qRT-PCR for MrgprA1, salusin-β, vomeronasal 1 receptor 159 (Vmn1r159), olfactory receptor 478 (Olfr478), Olfr960, and Olfr1238 (Fig. 1C). Taken together, these results reveal that salusin-β and MrgprA1 is highly involved and is likely to be important in the KD5040-induced survival of midbrain DA neurons.

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